IMMUNE BODIES IN URINARY INFECTIONS WITH COLON BACILLI AND TREATMENT BY INOCULATION.*

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STUDIES on infections of the urinary tract with especial reference to bacilli of the colon group have been made by a number of observers, notably by Rovsing, Hallé, Krogius, Brown, Dudgeon, and Wilson.

General conclusions reached by the writers are: first, the colon bacillus is the most common infection of the urinary tract; second, these infections may be acute, or chronic, the latter sometimes lasting for years; third, the most common causes are obstruction to the outflow of urine, pressure upon some part of the urinary tract or organs, and invasion of these structures from the intestinal tract, from the exterior through the urethra, or from the blood; fourth, it is common to find strains of colon bacilli in these cases differing markedly in their biological reactions; fifth, agglutination is rarely obtained even in low dilutions (Dudgeon) and is of little or no value for diagnosis.

Among others, Dudgeon[1] has studied cases of this kind with respect to the opsonic content of the serum and the treatment with killed bacteria. He found as a rule a low opsonic index, which by proper inoculation could be raised, and in some cases this rise was accompanied by improvement in the condition of the patient. In some cases, too, the urine improved greatly though this did not always correspond to the clinical improvement. Relapses were liable to occur. He concludes that the results justify the use of the autogenous bacillus.

Dudgeon did not find any appreciable bactericidal action of either normal or patient's serum upon strains of colon bacilli isolated from the urine. Neither did he find any increase in the bactericidal power of anticolon horse serum.

The results reported in this paper deal chiefly with the properties of serum toward the infecting organism, and with the inoculation

* Received for publication February 19, 1909.

[1] *Lancet*, 1908, 86, p. 615.

treatment. The study centers round certain of the cases whose sera presented unusual features with respect to their bactericidal action. The possible identity of the immune bodies concerned in phagocytosis and bacteriolysis, and the reciprocal adaptation of bacteria and host, are also discussed.

In eight cases of urinary infection the infecting bacilli have been isolated, and the cultural properties studied as well as the effect of normal and homologous serum upon the organism in question. The bacteria vary much both in morphology and cultural reactions. Some are short and plump; others slender and long; some grow profusely on ordinary media, others give a scant growth; all grow under anaerobic and aerobic conditions; two strains give a more profuse growth anaerobically than aerobically. Some coagulate milk, others do not; all acidify it. They vary much in the rapidity with which they ferment sugars. All produce indol. Two strains are hemolytic, producing clear zones on blood-agar plates; the others are not hemolytic.

The important features of the cases are here given.

The first case studied is of unusual interest and is considered somewhat at length.

Case 1.—Woman, age 24. Married and has one child. The patient had the usual diseases of childhood, most of them being very severe, but with no serious complications. Menstruation began at 13; during the first year the periods were irregular and accompanied by severe pain. Since then they have given her no trouble. No hereditary disease of any kind is known to exist in the family.

The patient gives a history of turbid urine since girlhood and years ago was told by physicians that she had "bladder trouble." About six years ago she noted that the urine was bloody after excessive physical fatigue and nervous strain. This disappeared in a few days, but a year later she had a recurrence following fatigue. Four years ago, at intervals of several months, she had four attacks of severe cramplike pains in the abdomen about the median line which required opiates for relief. No blood appeared in the urine at the time of these attacks, and no chill, fever, or vomiting accompanied them.

Three years ago the patient was married. Soon afterward she had an attack similar to asthma lasting for several weeks. A few weeks later there appeared in the urine, which had been turbid at intervals, white ropy streaks and on cystoscopic examination a probable diagnosis of renal tuberculosis was given and she was advised to go to California for the winter, which she did. While there she became pregnant and noted that at intervals the urine was bloody. The hematuria continued during pregnancy. A forceps delivery was necessary; the child was normal and no complications occurred. Following pregnancy the hematuria recurred at intervals as before and has continued up to the present time becoming, on the whole, more frequent and more

severe. During February, 1907, she took a severe cold and was in bed for several weeks during which time she lost much weight and strength. The hematuria at times was severe. A year ago, and also more recently, X-ray examination failed to reveal any evidence of stone in the urinary tract. Examination of the urine for tubercle bacilli by staining and also by inoculation of the sediment in guinea-pigs has constantly given negative results.

In June, 1907, she entered the Presbyterian Hospital in the service of Dr. Billings. The lower pole of the right kidney was easily palpable; otherwise physical examination revealed nothing abnormal. The blood gave 3,500,000 reds, 8,600 whites, and 57 per cent hemoglobin. Temperature normal. The urine was constantly turbid; some days it was bloody, at other times straw-colored. Red corpuscles together with a considerable number of leucocytes and bacteria were always present. Casts were never found. Albumin was present in small amount; no sugar.

A few days after entrance a cystoscopic examination was made by Dr. J. C. Webster and both ureters catheterized. The bladder was normal. A small clot of blood appeared at both ureteral openings. The right ureter appeared to be thickened and the catheter entered with considerable resistance but did not meet a definite obstruction. No urine was obtained from this ureter. Urine flowed freely from the left ureter. It was turbid, acid in reaction, contained a few leucocytes, red cells, epithelial cells, and a small amount of mucin; also bacteria identical with those obtained previously from the bladder and which will be described later.

A few days later a right nephrotomy was made. The capsule was normal. The pelvis and ureter were thickened and the catheter met an obstruction in the ureter a short distance below the pelvis. There was no evidence of tumor, calculi, or tuberculosis. Fluid from the pelvis of the kidney showed bacteria identical with those obtained from the bladder and the left ureter. A small piece of kidney tissue, removed at the operation, microscopically showed some degeneration of the cells and between many tubules the blood vessels were dilated. No blood was seen outside the vessels. The kidney was decapsulated, fixed, and drained. The tube remained in the kidney for several weeks and argyrol injected into the tube passed freely through the ureter into the bladder. The opening later closed and the tube was removed. A few days later blood appeared in the urine in quantity sufficient to color it distinctly red. For three weeks the urine was free from blood, then suddenly reappeared and has continued almost constantly. The patient's general condition remained about the same; the hemoglobin has remained low (about 60 per cent).

From the urine, from both ureters, and from the pelvis of the right kidney a colon-like bacillus was obtained in pure growth on the plates. Smears of the urine showed also a few Gram-positive bacilli often occurring in long chains. The colon-like bacillus was found constantly in the urine for several months. The ureters were catheterized just before the operation and again seven months later and this same organism obtained each time. No doubt the same bacillus has been the constant inhabitant of the urinary tract during many years.

The bacillus has the following properties:

Morphologically it resembles *B. coli*. It is a Gram-negative, non-motile bacillus, rather slender, and not infrequently forms short curved threads. On all media it grows less luxuriantly than *B. coli* and produces gas slowly in glucose with the ratio $H : CO_2 = 2 : 1$. It also produces gas in maltose, levulose, saccharose, lactose, and inulin. It permanently acidifies milk in 36 to 48 hours, but coagulation does not occur upon standing, even for many weeks. It produces indol and nitrites in Dunham's peptone medium; it grows freely in urine, causing uniform turbidity and later considerable sediment. It does not produce hemolysis on blood-agar plates. For animals it has about the same pathogenicity as *B. coli*. One agar slant kills a guinea-pig as a rule in 24 hours.

This bacillus, therefore, differing as it does from *B. coli*, in not coagulating milk and growing less luxuriantly on various media, may be considered a modified colon organism.

With a view to inoculation treatment, the opsonic index of the patient for this bacillus was obtained from time to time. Two determinations gave indices of 0.7 and 0.4 respectively (Chart 1). After the injection of 50 million bacilli killed by heat the index promptly rose. The injections were continued at intervals of about one week and the index, as shown in the chart, continued to remain high. Heated serum (Chart 1) and the dilution method also gave high indices, as a rule above 2, the curves on the whole corresponding very well. The difference between the phagocytosis in the normal and the patient's serum was so striking that it could readily be detected by merely inspecting the slides. The index for other bacteria was normal or slightly below (Table 1). The specificity of the opsonin

TABLE 1.

OPSONIC INDEX OF PATIENT'S SERUM FOR OTHER BACTERIA COMPARED WITH THAT FOR AUTOGENOUS BACILLUS.

Organism	Index	Index for Autogenous Bacillus
Typhoid	1.10	2.60
Streptococcus pyogenes	0.96	1.30
B. coli	0.61	1.30
Staphylococcus aureus	0.70	1.85
Pneumococcus	0.93	1.85
Tubercle Bacillus	0.75	2.60
B. coli (from a case of pyelitis)	1.30	2.20

is evident. Even for typical colon bacilli which differ but slightly in cultural characteristics the index is much lower.

In determining the opsonic index it was noted that when unheated serum was used there was distinct evidence of bacteriolysis in the

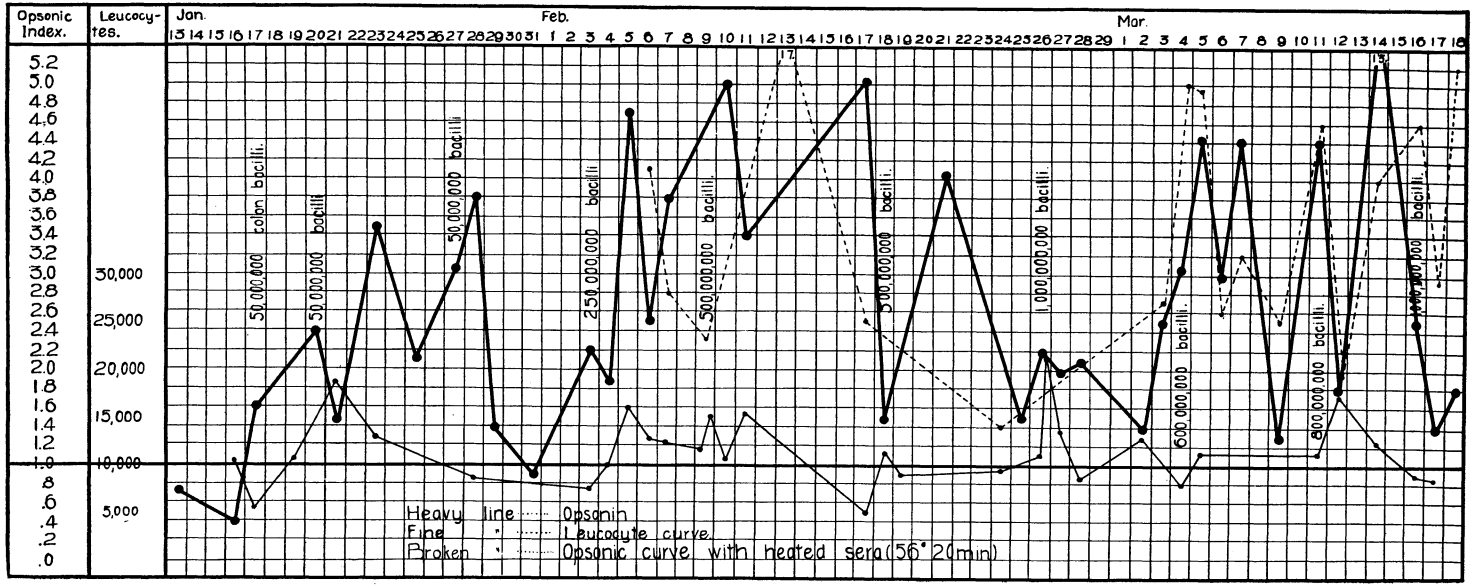

CHART 1.—Leucocyte curve and opsonic index with unheated and heated serum in Case 1.

tube containing normal serum, whereas in the tube containing the patient's serum little or no such change occurred. Under the microscope the process of solution of the bacilli in normal sera could be readily observed, while the patient's serum was quite inert. Also on comparing the lytic property of normal and patient's serum by the plate method, it was found that the former rapidly destroyed the organism while the latter had little or no lytic power. Heating normal serum to 56° C. for 20 minutes destroyed the lytic effect and heating the patient's serum produced no change. Table 2 is arranged to

TABLE 2.

LYSIS OF COLON-LIKE BACILLUS.

	No. of Colonies at Intervals			
	At Once	2 Hrs.	5 Hrs.	18 Hrs.
Normal Serum A, Unheated	100	0	0	0
" " A, "	5,000	0	0	0
" " B, "	4,000	2	0	0
" " C, "	4,000	0	0	0
" " D, "	3,500	80	0	0
Normal Serum A, Heated 56° 20 min.	5,000	4,500	5,000	8,000
" " B, "	2,000	2,000	2,500	2,000
" " C, "	2,000	1,800	1,800	30
NaCl Solution	4,000	2,000	1,000	1,000
Feb. 10, Patient's Unheated Serum	5,000	3,500	500	....
" 24, " " "	2,000	1,500	2,000	500
Mar. 3, " " "	4,000	800	1,000	100
" 17, " " "	2,000	2,000	2,500	400
Feb. 17, " Heated Serum	5,000	6,000	6,000	6,000
" 24, " " "	2,000	2,000	1,000	2,000
Mar. 17, " " "	2,000	2,500	2,000	600

show the effect of heated and unheated sera upon the growth of this organism. Four different normal sera were tested and all acted alike. The lytic power of the patient's serum toward this organism was tested at intervals for a period of six weeks with the same results. A small amount of normal serum reactivated for lysis the normal heated serum but not the patient's heated serum. It appears, therefore, that in the patient's serum there is little or no specific lytic amboceptor for this particular organism, while in normal serum this body is present.

The lytic effect of the patient's serum was tested upon typical colon and typhoid bacilli and compared with the normal sera in this respect (Table 3). The patient's serum is as lytic for these organisms as normal sera.

TABLE 3.

LYSIS OF COLON AND TYPHOID BACILLI.

	No. of Colonies at Intervals			
	At Once	2 Hrs.	5 Hrs.	18 Hrs.
B. coli (typical) + Patient's Serum	6,000	1,500	75	0
B. coli " + Normal Serum A	8,000	3,000	200	46
B. coli " + " " B	8,000	4,000	600	10,000*
B. typhosus + Patient's Serum	3,000	3	0	0
" + Normal Serum A	4,000	5	0	0
" + " " B	2,000	15	0	0
" + " " C	6,000	900	200	0
" + NaCl Solution	6,000	3,000	2,000	1,000

* This serum was obtained from a person who a few months previously had an operation for acute appendicitis. The hemolytic property of his serum was also less than normal.

The lytic power of the serum upon goat, rabbit, and guinea-pig corpuscles was normal.[1]

This serum differs, then, from normal serum in that it has practically no lytic effect upon the autogenous bacillus; it possesses, however, normal lytic power for other even closely related bacteria and also for red corpuscles.

Owing to the rapid lytic action of normal unheated serum upon the bacillus accurate determination of opsonin consequently could not be made. With heated sera this difficulty was removed, because, as shown, heated normal and patient's sera have practically no lytic power. Heating to 56° C. for 20 minutes markedly decreased, but did not entirely prevent phagocytosis in either normal or patient's serum.

In order to show more clearly the relation of phagocytosis to bacteriolysis in heated and unheated serum, the process was followed by making smears of mixtures in the incubator at frequent intervals and determining the amount of phagocytosis. Chart 2 shows a series of curves obtained in this manner. Counts were made at 3, 6, 10, 20, 60, and 120 minute intervals of the number of bacilli in 50 leucocytes.

Curve *A* is lytic normal serum. Phagocytosis proceeds rapidly in the first few minutes, but owing both to intra- and extra-cellular lysis the curve falls. The bacilli in the stained preparations were disintegrating both inside and outside the leucocytes and at the end of two hours had largely disappeared. Curves *B*, *C*, and *D* represent

[1] I have to thank Dr. Preston Kyes for these experiments.

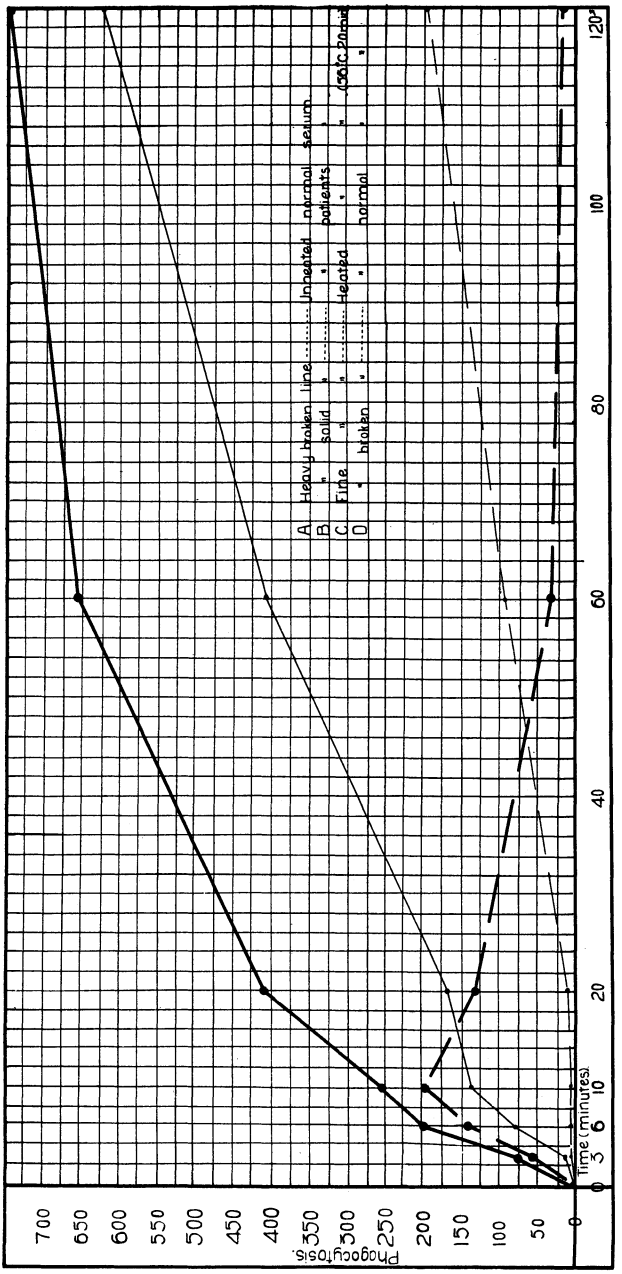

CHART 2.—The curves represent the average number of bacteria taken up by 50 leucocytes at varying intervals of time, using unheated and heated normal serum and serum of patient in Case 1.

the process of phagocytosis independent of bacteriolysis. The space between *B* and *C* represents the loss of opsonic effect by heat in the patient's (immune) serum. The space between the normal line and *C* represents the thermostable opsonic element. In heated normal serum the opsonic content was low as shown by curve *D*. At the end of 120 minutes, however, the phagocytosis is considerable. It is interesting to observe that here the bacilli within the leucocytes as well as those outside remained undigested, while in the unheated normal serum the digestion goes on rapidly, both within and without the cells. It would appear from this that intracellular digestion of the bacilli depends upon the action of the serum either upon the bacteria before phagocytosis occurs or upon the leucocytes. In curves *B* and *C* the leucocytes at the end of 120 minutes were well filled with bacilli which show only slight evidence of digestion. Undoubtedly intracellular digestion was going on, as will be shown later, but the process was so slow that phagocytosis kept the leucocytes well filled with bacilli but little injured so far as the staining reaction indicated.

This series of curves shows clearly the importance of the time interval in determining the opsonic index as well as the necessity of knowing whether or not a serum is lytic. For instance, in using unheated sera in this case the longer the interval of time the greater will be the opsonic index; with heated serum, since the curves are more nearly parallel, the index at varying intervals will not vary greatly. With lytic sera, especially unheated, indices obtained within the first few minutes will on the whole be much more trustworthy.

Reactivation experiments with the patient's heated serum and with normal heated serum gave definite results pointing to the co-operation of a thermostable and a thermolabile substance in opsonification. A small amount of normal serum added to the heated sera increased phagocytosis both in the patient's serum and in normal serum.

Table 4 shows the combined action of the two bodies in opsonification and also indicates that the thermostable body, not being removed by washing, is securely bound to the bacteria. The thermolabile body on the other hand is easily removed by washing. These facts hold true for both normal and immune serum.

The agglutinating power of the serum was tested upon the homol-

ogous bacillus at various intervals. These experiments were made at the same time as the dilution experiments for phagocytosis, every day or every second day for a period of two weeks. Both macroscopic and microscopic methods were used, but at no time was any specific agglutination observed. The bacillus was slightly agglutinated in dilutions 1–10 and 1–20 in both normal and patient's serum. In one or two instances the homologous serum seemed to be even less agglutinative than the normal.

TABLE 4.

REACTIVATION OF HEATED IMMUNE SERUM.

1. Bacilli 0.3 c.c. + Patient's heated serum 0.1 c.c. + NaCl Sol. 0.1 c.c.
2. " 0.3 c.c. + " " " 0.1 c.c. + Normal Serum 0.02 c.c. + NaCl Sol. 0.08 c.c.
3. " 0.3 c.c. + " " " 0.1 c.c. + NaCl Sol. 0.02 c.c.
4. " 0.3 c.c. + NaCl 0.1 c.c. + Nor. Serum 0.02 c.c. + NaCl Sol. 0.08 c.c.

Incubated 45 minutes; centrifuged 30 minutes. Bacilli then suspended in 0.3 c.c. NaCl and mixed with leucocytes and serum as follows:

		Phagocytosis in 50 Leucocytes (20 minutes).
1.	Bacilli 0.3 c.c. + Leucocytes 0.1 c.c. + NaCl Sol. 0.1 c.c.	 205
2.	" 0.3 c.c. + " 0.1 c.c. + " " 0.1 c.	191
3.	" 0.3 c.c. + " 0.1 c.c. + Nor. Ser. 0.02 + NaCl Sol. 0.08 c.c.	...563
4.	" 0.3 c.c. + " 0.1 c.c. + NaCl Sol. 0.1 c.c.	 0

The importance of phagocytosis in destroying bacilli of this type was clearly brought out by the experiment, the results of which are shown in Table 5. The patient's unheated serum and washed

TABLE 5.

INTRAPHAGOCYTIC DESTRUCTION OF COLON BACILLI.

	No. of Leucocytes in 1 cu. mm.	No. of Colonies on Plates			
		At Once	2 Hrs.	5 Hrs.	18 Hrs.
1. Unheated Serum.....	.....	2,000	1,500	2,000	500
2. Washed Leucocytes.....	30,000	2,000	800	500	600
3. Washed Leucocytes + Serum.....	400	2,000	2,000	600	3,000
4. " " + "	4,400	2,000	200	600	5,000
5. " " + "	16,000	2,000	10	0	0

leucocytes by themselves have no prompt destructive effect on the bacilli. When mixed the result depends upon the number of leucocytes present. If this number is low (400) the bacilli are not destroyed; by concentrating the leucocytes (16,000) they are destroyed, thus showing that the bacilli after being taken up by the leucocytes are killed even though they microscopically show little evidence of intracellular digestion. In this connection may be mentioned some

experiments the results of which appear to indicate differences in the phagocytic activity of the patient's leucocytes and of normal leucocytes. Dudgeon and Shattock[1] found as a rule an increased activity of the immune phagocytes, but in certain conditions it was normal or subnormal.

Table 6 shows the result of crossing normal and patient's leucocytes with normal and patient's sera. Four experiments were made,

TABLE 6.

COMPARATIVE PHAGOCYTIC ACTIVITY OF LEUCOCYTES.

						No. of Bacilli in 50 Leucocytes	
Normal Leucocytes	A	+ Patient's	Serum	+	Bacilli	278	376
" "		+ Normal	" A	+	"	98	
Patient's "		+ Patient's	"	+	"	296	374
" "		+ Normal	" A	+	"	78	
Normal "	B*	+ Patient's	"	+	"	148	192
" "		+ Normal	" B	+	"	44	
Patient's "		+ Patient's	"	+	"	188	250
" "		+ Normal	" B	+	"	62	
Normal "	C	+ Patient's	"	+	"	134	174
" "		+ Normal	" C	+	"	40	
Patient's "		+ Patient's	"	+	"	254	446
" "		+ Normal	" C	+	"	192	

* Experiment with leucocytes B repeated at another time gave substantially the same results.

using leucocytes from three different normal persons. The number of bacilli taken up by normal leucocytes A and patient's leucocytes were about the same. Normal leucocytes B and C contained less bacteria than the patient's leucocytes. This may be interpreted in one of two ways. The number of bacteria as determined by count is not necessarily the number taken up by the leucocytes because of the process of intracellular digestion which is an important factor in the case of this bacillus. Consequently the data given in Table 6 may signify either an increased phagocytic activity of the patient's leucocytes or a less rapid process of intracellular digestion in these leucocytes as compared with normal leucocytes or both.

Case 2.—This case was a chronic urinary infection of at least five years' standing, occurring in a young man. The urine was constantly turbid, contained leucocytes in small numbers but never any blood. A short, plump, non-motile, Gram-negative bacillus was obtained in pure growth; it produced gas readily in the various sugars, acidified but did not coagulate milk, and formed indol. Injections of the autogenous dead bacilli in doses of 400 million to one billion were given every 5 to 7 days. They

[1] *Lancet*, 1908, 86, p. 618.

gave rise to definite local and general reactions, manifested by localized tenderness, redness, and swelling at the point of injection, and by fever, leucocytosis, irritation of the bladder, malaise, and soreness in the muscles and joints generally.

The opsonic index (Chart 3) was high at first and rose still higher following the inoculation treatment. As a rule a drop occurred in

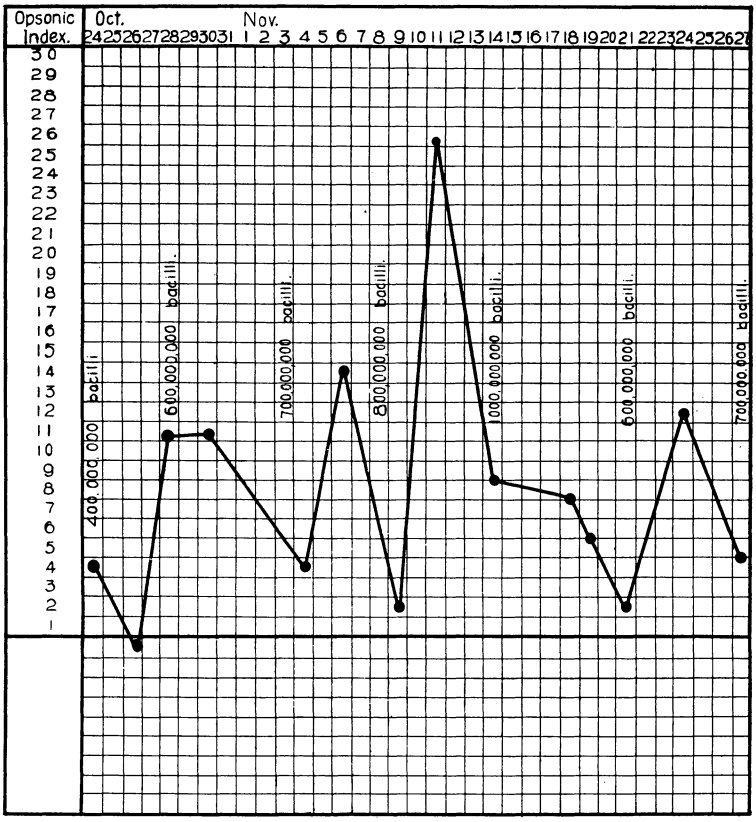

CHART 3.—Opsonic index of Case 2 obtained with unheated serum and homologous bacilli.

the opsonic curve after the injection, followed by a marked rise. A striking feature was the comparative insusceptibility of this organism to phagocytosis, necessitating the use of dense suspensions of bacteria to obtain an appreciable phagocytosis. The organism was not highly virulent for animals. The opsonic index for another strain of *B. coli* was normal.

Lysis of this bacillus was clearly evident under the microscope. By the plate method it was shown that while both were distinctly lytic, normal serum was more so than the homologous serum. This continued so in spite of repeated injections with the dead bacilli. In marked contrast to Case 1, serum heated to 56° C. for 20 minutes seemed to lose its opsonin entirely for this bacillus. Tests made over a period of several weeks gave uniform results. By adding small amounts of normal serum to the heated sera it was possible to reactivate both the patient's serum and normal serum for opsonification. For bacteriolysis slight reactivation was obtained in both normal and patient's sera.

Case 3.—In this case, which was one of long-standing infection of the urinary tract with a typical colon bacillus, probably associated with stone, the opsonic indices, taken five times on alternate days, were 0.4, 0.2, 0.2, 0.6, and 0.7. Using heated sera they were approximately the same. The lytic power of the serum taken twice during this period was practically normal. Here the unusually low opsonic content was associated with the normal amount of lytic substance. No inoculations were made.

Case 4.—For the last two months of pregnancy the patient had turbid urine, frequent urinations, and chills and fever. A pure growth of a colon-like bacillus was obtained from the urine associated with a large number of leucocytes. The opsonic index was about normal. An injection of 500 million dead bacteria was given. At this time birth occurred. The indices on the following alternate days were 1.8, 3.0, 3.5, 4.3, 2.0, 8.5, 2.1, 3.8, and 3.3. The bacteriolytic action of the serum was normal.

Case 5 was one of urinary infection of several months' standing with a non-hemolytic, Gram-negative, non-motile bacillus which acidified and coagulated milk and fermented the various sugars with the production of gas. The opsonic index taken at three different times was 1.1, 1.3, and 1.2. The bacillus was equally susceptible to the lytic action of both normal and homologous sera, even in very high dilutions. No specific agglutination was observed.

Case 6 was one of long-standing urinary infection occurring in a man who gave a history of syphilis 22 years back and gonorrhea 15 years back, complicated with stricture. Eighteen months before coming under observation the patient developed a more or less general acute painful arthritis involving especially the right foot, and later both elbows, hands, and the left foot. He also had symptoms pointing to locomotor ataxia and from the prostate were obtained forms suggestive of gonococci. The temperature was as a rule normal. The urine was constantly turbid and contained numerous pus cells and bacilli. Urination was frequent and difficult. The organism present in the urine was a Gram-negative, non-motile bacillus often slightly curved and staining irregularly. Phagocytosis was not appreciable in the urine but occurred freely in the presence of serum. It did not acidify or coagulate milk, produced a trace of indol, fermented mannite and dextrin, but not glucose or galactose. In aerobic cultures growth as a rule was scant, both in plain and special media, and at times no growth whatever occurred. In anaerobic cultures growth always took place and was much

more profuse. On blood plates the colonies were from 0.5 mm. to 1 mm. in diameter, surrounded by a wide clear zone of hemolysis. There was no odor.

Both normal and homologous sera were equally highly lytic for this bacillus while salt solution was inert. Slight agglutination occurred in two hours at 1–40 in the patient's serum but none in the control. The opsonic index taken from time to time varied from normal to 2 or slightly above.

For a period of many months the patient received injections of the dead bacilli about once a week in doses varying from 200 million to one billion. He received two injections of dead gonococci early in the treatment. A slight reaction occurred. The reactions following the injection of bacilli were at times intense. Marked improvement took place the details of which will be discussed more fully later. Here attention is called to the anaerobic character of the bacillus, its marked susceptibility to lysis by both normal and homologous sera, and the slight agglutinating property of the patient's serum, its opsonic power being higher than normal.

Case 7 gave a history of an indefinite "grippe" infection several months previously. Improvement followed, but later there developed aching pains and stiffness in various joints, particularly the knee. No marked swelling of the joint occurred at any time and no urinary symptoms were noticed. The urine (catheterized specimen) upon examination showed the presence of a small number of pus cells, some epithelium, but no red cells or casts. A very small, irregularly staining, Gram-negative, non-motile bacillus was found in smears made from the sediment and on blood-agar plates small colonies appeared after from 48 to 72 hours. These were so small that they might easily escape detection on the plates were it not for the clear wide zone of hemolysis about them. At times in aerobic cultures no growth whatever occurred in the plates. Under anaerobic conditions, however, the growth was more rapid and profuse, and as a rule produced a wider zone of hemolysis. All attempts to grow this organism on media other than blood media were futile. The organism was readily killed by heat, and about once a week from 100 to 500 million bacilli were injected subcutaneously. A definite reaction followed, manifested by local swelling, tenderness, redness, slight fever, and leucocytosis with increased pains in the joints and muscles generally. In this case no vesical irritation followed these injections as occurred in some of the other cases. Marked improvement took place, the bacilli gradually decreased in numbers in the urine, and the pains and stiffness in the joints improved. The bacilli growing in clumps both in the urine and in media, attempts to determine the opsonic index were not satisfactory. The percentage index gave 0.66 and 1.8 at two different times. In this case considerable spontaneous phagocytosis occurred much the same as in the case of influenza bacilli.

Case 8 is one of long-standing tuberculosis of the genito-urinary system with secondary colon infection in a man, 31 years of age. Eight years previously the infection began as an acute painful swelling of the right testicle. Later the glands in both groins became involved. These were opened and drained and the testicle removed. For two years the patient did not do well, and after two further operations he went to New Mexico where he improved greatly. But the following year there was a recurrence of the disease in the groin, and a year later he began to have pain in the small of the back on both sides and also pain on urination. At first the urine was clear but soon it became turbid and he was obliged to urinate frequently. Up to this time his general health remained fairly good. Now, however, he rapidly lost in strength and weight and remained in bed a large part of the time. The pain in the back and in the region

of the bladder was intense and he was not able to lie on his side. In the spring of 1907 right nephrotomy was made but no improvement took place. In the fall of 1907 he came to the Presbyterian Hospital in the service of Dr. Billings. The urine contained constantly a large amount of pus, often considerable quantities of blood, and numerous tubercle bacilli. Urination occurred every 30 minutes and was accompanied by severe pain. Walking, sitting, and lying on either side were painful and he spent most of the time on his back. The ophthalmo-tuberculin test gave a positive reaction. In addition to the tubercle bacilli there was an abundant growth of a typical colon bacillus in the urine. Simultaneous injections were given of Koch's new tuberculin (1/1,000 mgm.) and of killed autogenous colon bacilli. The tuberculo-opsonic curve followed a fairly typical curve, showing a slow but gradual rise in response to the injections. Injections of the dead colon bacillus in doses of 500 million were given once a week and caused sharp reactions both local and general; after three injections the bacilli had entirely disappeared from the urine. The colon opsonic index which was low at first (0.8) rose to between 2 and 3 and remained high. The patient gradually improved. The colon injections were discontinued and the tuberculin given weekly continuously for a year. During this time the patient gained over 20 pounds in weight and became much stronger in every way. The pains grew less, urination occurred about once in two hours, and he was able to go about and do light work. One year later his tuberculo-opsonic index was 2.1 and the colon bacilli were not found in the urine. Tubercle bacilli were still found and pus cells were numerous, though the centrifuged sediment was not one-tenth as much as a year previously. The opsonic and temperature curves are given in Chart 4.

SUMMARY AND GENERAL CONSIDERATIONS.

In a case (Case 1) of long-standing infection with a colon-like organism the specific opsonic index, low at first, upon inoculation rose and remained high. The patient's serum had no bacteriolytic power for the homologous bacillus and no change in this respect followed the inoculations. Normal sera for the same organism were highly lytic. For other closely related bacilli (*B. coli* and *B. typhosus*) and for red corpuscles of various animals, the patient's serum was normally lytic. It was possible to reactivate the patient's heated serum for opsonification but not for bacteriolysis, while normal heated serum could be reactivated for both opsonification and bacteriolysis. Specific agglutinins were not present in the patient's serum.

In a second case (Case 2) a high opsonic content was associated with a subnormal lytic power. Normal serum and the patient's serum could be reactivated for both bacteriolysis and opsonification. Specific agglutinins did not appear.

In a third case (Case 3) an unusually low opsonic index was associated with normal lytic activity.

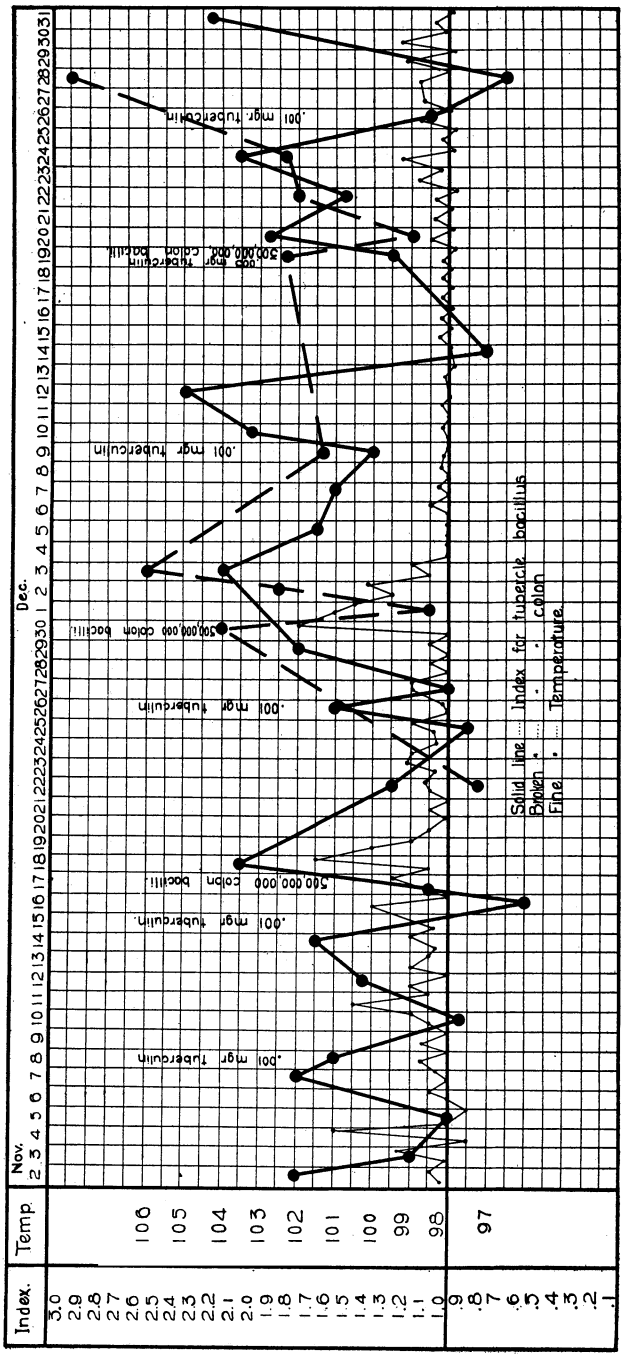

CHART 4.—The temperature curve and opsonic index for tubercle bacillus and colon bacillus in Case 8.

These facts appear to be important in relation to the question of the identity of the lytic amboceptor and the stable opsonic body. In Cases 2 and 3 one may explain the results by either one of two assumptions. First, one may assume that opsonins and lysins are different substances and the one may vary independently of the other; or, second, the opsonin and lysin may be one and the same and on account of certain changes the microbe has become immune to bacteriolysis or opsonification as the case may be. Either of the above assumptions may explain, perhaps equally well, the facts as observed in these two cases, but they do not explain equally well the facts in Case 1. Here the serum increased markedly in its specific opsonic property as a result of inoculation, whereas it remained as inert as salt solution in its specific bacteriolytic power, while the bacillus was highly susceptible to bacteriolysis by normal serum. On the basis of the first assumption, namely, that lysins and opsonins are different substances and may vary independently, the facts may be explained thus: the opsonins increased and remained high; the lysins normally present when the infection began were absorbed and for some reason the body did not react to produce them in appreciable quantities. On the basis of the second assumption—that opsonification and bacteriolysis depend on the same substance and the microbe undergoes specific changes—it is impossible to understand how the bacillus could acquire the property of resisting bacteriolysis and at the same time not only retain but vary in its susceptibility to phagocytosis as the facts indicate. We might say that in Case 1, for example, the bacillus had immunized itself against the lytic action of the patient's serum. But from what is known of the principles of immunity it should also be immune to the lytic action of serum from all individuals of that species. This is not true.

The facts are better explained by assuming that the microbe gaining access to the individual causes specific reactions in the serum to meet which the microbe specifically adapts itself. The microbe thus immunizes itself against an abnormal individual and may be still susceptible to the serum of a normal person. This reciprocal adaptation continuing for a long period may give rise to profound changes on the part of both microbe and host. Attention is called to the fact that the cases here reported are very chronic, Case 1, for

instance, dating back at least five years and possibly ten or more years. It would not be surprising, therefore, to meet with changes in the serum in such cases, very different in character from the changes ordinarily met with in the more acute infections.

OPSONINS IN THE URINE.

Normal urine when added to washed leucocytes and colon bacilli does not cause phagocytosis. If serum is added to this mixture the leucocytes take up the bacteria as freely as if salt solution replaced the urine. Urine, therefore, does not inhibit phagocytosis. In Case 1 there were constantly present in the urine, even when blood was absent, numbers of polynuclear leucocytes and albumen, but phagocytosis occurred only to a slight extent even when blood was present in large amounts and the bacilli formed a dense suspension. In the test-tube bloody urine added to washed corpuscles and bacteria and allowed to incubate for one hour caused an appreciable amount of phagocytosis. An experiment made with a specimen of such bloody urine showed slightly less phagocytosis than in a 1:100 dilution of the patient's serum taken at the same time. In Case 2 the urine, which was free from blood and albumen, was tested for opsonin at a time when the opsonic index of the blood was very high (11.1). A few leucocytes and many bacilli were present in the urine but there was no appreciable phagocytosis, either at the time it came from the bladder or upon standing in the incubator. Serum added to such urine caused the leucocytes to freely ingest the bacteria. These leucocytes, however, were not as active as fresh leucocytes obtained from the blood and many were undergoing disintegration. It appears, therefore, that the urine, from certain cases whose blood possesses a high opsonic content for the infecting organism, may contain practically no opsonin for the same organism. On the other hand at times, as was noted in certain of the cases, an appreciable phagocytosis of bacilli occurred in the urine. In the cases in which this was observed, however, the bacilli were far more phagocytable than the bacilli from the cases mentioned above.

In Case 8 the urine contained constantly large numbers of tubercle bacilli, but it was exceptional to find one within a leucocyte. At times there seemed to be slightly more phagocytosis than at others.

After marked improvement had occurred and the tuberculo-opsonic index was high (2.1) the relation of bacilli and leucocytes did not change. These findings are in accord with those of Dudgeon[1] and also with those of Opie[2] who found frequently little or no opsonin in many inflammatory exudates.

INOCULATION OF DEAD COLON BACTERIA AND THE REACTION PRODUCED THEREBY.

In view of the fact that the bacilli isolated from cases of urinary infection usually differ more or less from one another, homologous bacilli were used in the cases here reported. The bacilli in pure growth were killed by heating to 60° C. for 30 minutes. This was sufficient in every instance to kill the bacilli as shown by the control culture. Fresh suspensions should be used. For the first injection not over 200 million bacilli should be given; this may then be increased until a suitable reaction is obtained. It is necessary to do this for the reason that the strains isolated from the various cases differ in the strength of reaction which they produce. Three hundred million bacilli of one strain may produce as strong reaction as 600 million organisms of another strain. As has been stated the bacilli of various strains differ considerably in their size and this may partly explain variations in effect when the number of organisms is used as a standard. Other factors also undoubtedly come in. The dose, therefore, for organisms of this type may vary from 200 million to one billion.

The local reaction consists of tenderness, redness, and swelling over an area several centimeters square. This begins in one or two hours following the injection, reaches the height in about 12 to 18 hours, and disappears in 48 to 72 hours. A general reaction occurs, manifested by leucocytosis, fever, sometimes headache, general malaise, aching in muscles, bones, and joints, and often, but not in all cases, irritation of the bladder indicated by a frequent desire to urinate. This latter symptom was especially marked in Case 2. Because of the fact that the strains of bacilli vary so markedly in their cultural properties, and especially in their serum reactions, it does not appear reasonable to use stock solutions of dead colon bacilli for purposes of treatment or for diagnosis.

[1] *Loc. cit.* [2] *Jour. Exp. Med.*, 1907, 9, p. 515.

It would be folly to attempt to draw definite conclusions concerning a therapeutic question from the series of cases here given. Suggestions only may be made and the facts taken for what they are worth. In one case only (Case 8) did the bacilli entirely disappear from the urine while the case was under observation. In most instances the condition of the urine improved as indicated by the number of leucocytes and bacilli, but did not entirely clear up, at least while the patient was under observation. The result in Case 6 was striking. The patient, following injections of about 500 million dead bacilli, went about his work. An hour later he had a most violent chill, followed by high fever (105.4° F.). After 24 hours the temperature became normal and the patient was able to go about as usual. This appeared to be the turning-point in his case. Severe and almost constant pain which he had had in his ankles for over a year appeared to have left him during the time he had the severe reaction, to return, if at all, only in very mild form. He quite rapidly improved, especially as to the arthritis, in a short time gained 30 pounds, and felt better than he had for years. The urine improved but did not entirely clear up. Injections were continued for nearly a year and the case is still under observation. Case 4 quickly improved after childbirth and the inoculations probably had little to do with the result.

In the cases which show a diminished bactericidal power of the blood, such as Cases 1 and 2, and also those with low opsonic indices, such as Case 3, it appears reasonable to attempt to combat the infection by using a method which will tend to raise the content of these immune substances in the body. In Case 1, for instance, where there was such a marked diminution, or a total absence of lytic substance, long-continued injection, though raising the opsonic index markedly, did not appear to have any appreciable affect in increasing the lytic power of the serum. This agrees with the results of Dudgeon who found that the opsonic indices were, as a rule, markedly affected by dead colon bacilli but anticolon serum had no more specific bactericidal effect than normal serum.

CONCLUSIONS.

In cases of urinary infections the bacteria of the colon group vary markedly in certain details. Consequently, for inoculation treat-

ment in these cases, the homologous germ should be used. The opsonic index may be raised by such inoculations. In certain cases the patient's serum may show a marked diminution or total absence of lytic bodies for the homologous bacilli, as compared with normal serum, while the specific opsonins may be present in abundance. In certain cases opsonins seem to play little or no part in the destruction of colon bacilli in the urine.

Opsonification, bacteriolysis, and agglutination in certain cases do not run parallel. The facts are most easily explained by assuming the existence of specific opsonins, lysins, and agglutinins.

NOTE.—Since this paper was written further results in some of the cases referred to and also in other cases treated later appear distinctly to favor the inoculation treatment. In Case 2 in which the inoculations were given weekly for nearly four months the pus and bacilli which were present in the urine constantly for over five years have entirely disappeared and the patient is now perfectly well. Case 1, clinically, has improved very much, but it is not known whether the bacilli have entirely disappeared. Case 6 improved markedly for a time but urethral strictures and symptoms of beginning locomotor ataxia have interfered with the results. A case of subacute pyelitis caused by a hemolytic colon-like bacillus apparently responded promptly to the inoculations and after five injections the urine was entirely free from pus and bacilli. Several other chronic cases now under observation all report improvement. Further detail of the treatment of these cases will be given later.